3-Bromopyruvate induces cytotoxicity, inhibits glycolysis and decreases migratory capacity in glioblastomas and colorectal cancer cell lines

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Introduction

The majority of cancer cells present an altered energetic metabolism resorting to aerobic glycolysis, even under aerobic conditions ("Warburg" effect) and this can be the basis for the development of new and more effective antitumor agents. 3-bromopyruvate (3-BP) is an alkylating agent putatively transported by the monocarboxylate transporters (MCTs), which targets cancer cell metabolism, and it has been demonstrated to be a powerful and specific antitumor agent either in *in vitro* or *in vivo* models. 3-BP inhibits tumor energetic metabolism, causing depletion of intracellular ATP, thus acting as a cytotoxic agent. Our published results and preliminary transport assays show that MCT1/4 and CD147 might play a key role in 3-BP uptake.

We investigated the effect of 3-BP in glioblastomas, a very aggressive cancer, and in colorectal cancer cell lines, which appears in top three ranking of the most frequent cancer types [1]. 3-BP cytotoxic effect was assessed at different extracellular pH (pHe) values and correlated with MCTs expression [2, 3]. 3-BP effect on migratory capacity and cell metabolism in the different cell lines was also determined.

References:
[1] Ferlay, J. *et al.* (2014). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *International Journal of Cancer*. 1-76.
[2] Azevedo-Silva, J. *et al.* (2015) The cytotoxicity of 3-Bromopyruvate in breast cancer cells depends on extracellular pH. *Biochem J*. 467: 247–258.
[3] Queiros, O. *et al.* (2012) Butyrate activates the monocarboxylate transporter MCT4 expression in breast cancer cells and enhances the antitumor activity of 3-bromopyruvate. *J Bioenerg Biomembr*. 44: 141-53.

Methods

Sulforhodamine B: The cytotoxic effect of 3-BP in glioblastoma and colorectal cancer cell lines was evaluated at basal conditions and at different extracellular pHs. The different cell lines were exposed in the assayed conditions to 3-BP for 16 hour and cell viability was determined after this period of time.

Wound healing assay: Cells were plated in 6-well plates and grown until total confluence. Two wounds were then created in the confluent cells by manual scratching. Cells were treated with ½ IC_{50} and IC_{50} values of the 3-BP, being untreated cells used as control. Photograph records were obtained cells at 0, 12 and 24 hour. Percentage of cell migration relative to time zero of the control was evaluated with the GraphPad Prism 5 software.

Glucose and lactate quantification: The different cell lines were exposed at ½ IC_{50} and IC_{50} values of 3-BP and lactate and glucose were measured in extracellular medium. As control, untreated cells were used. The obtained results were normalized to the protein biomass using SRB assay.

Western Blot: Protein extracts of different cancer cell lines were used to evaluate the MCT1 and MCT4 expression by Western-blotting. α-tubulin was used as loading control and the density of each band was determined with the software Image J (version 1.48, NIH).

Final Remarks

•3-BP presented cytotoxic effect to both glioblastoma and colorectal cancer cell lines, but with different sensitivities.

•3-BP was more cytotoxic at lower pHe in most cell lines assayed, according to its probable mechanism of transport by a proton-symport.

•The migratory capacity as well as energetic metabolism were inhibited by 3-BP, being this effect more evident when higher concentrations of the compound were used.

• In glioblastomas, MCT4 was more expressed in the most sensitive cell line, which also present a considerable expression of MCT1. As 3-BP is transported by MCTs, its cytotoxic effect probably be associated to the MCTs expression. However, in colorectal cancer cell lines no direct association was found.

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Results

Cytotoxic effect of the 3-BP in glioblastoma and colorectal cancer cell lines

Table 1. IC_{50} (μM) values for different cancer cell lines exposed to 3-BP.

		Glioblastoma Cell Lines			Colorectal Cancer Cell lines		
		U373MG	U87MG	U251MG	HCT-15	Caco-2	HT-29
Medium with $NaHCO_3$		64.71 ± 7.01	65.92 ± 6.95	95.72 ± 7.43	31.76 ± 6.28	234.15 ± 17.38	263,67 ± 74.89
Medium without $NaHCO_3$	pHe 6.6	26.18 ± 1.53	59.16 ± 5.00	43.55 ± 4.70	15.95 ± 6.07	ND	ND
	pHe 7.4	25.35 ± 3.68	75.86 ± 5.44	57.41 ± 5.47	19.55 ± 3.98	ND	ND

- The cell lines more resistant to 3-BP were U251MG and HT-29, in glioblastoma and colorectal cancer cell lines, respectively.
- 3-BP was more cytotoxic at pHe 6.6 than pHe 7.4. It was not possible determinate IC_{50} value at different pHe for Caco-2 and HT-29 in colorectal cancer cell lines.

Metabolic profile in glioblastoma and colorectal cancer cell lines

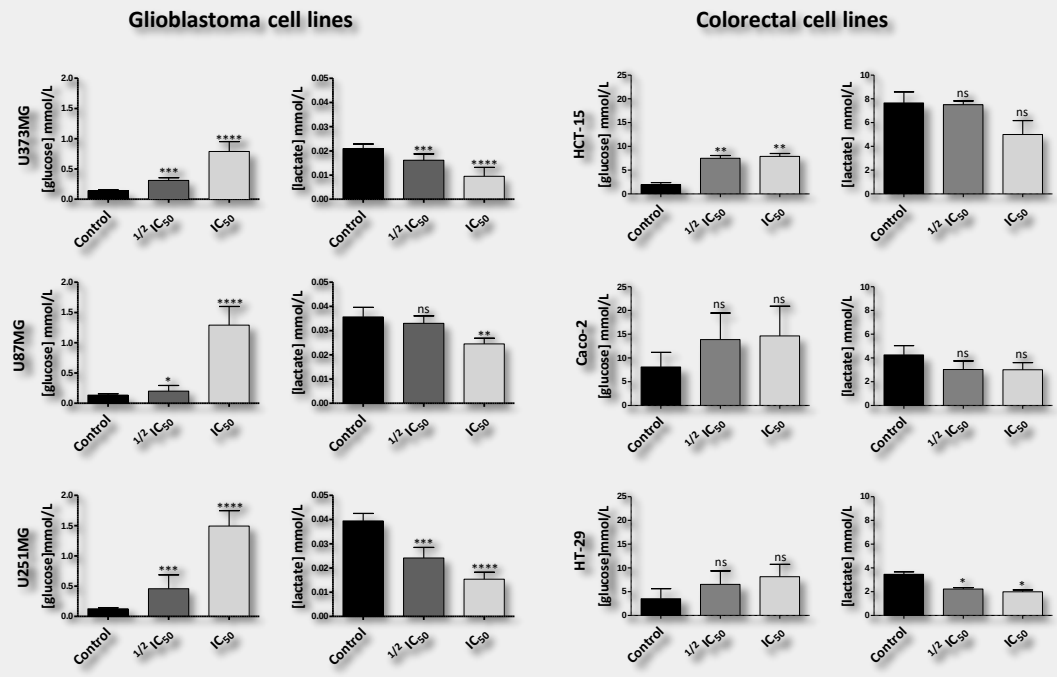
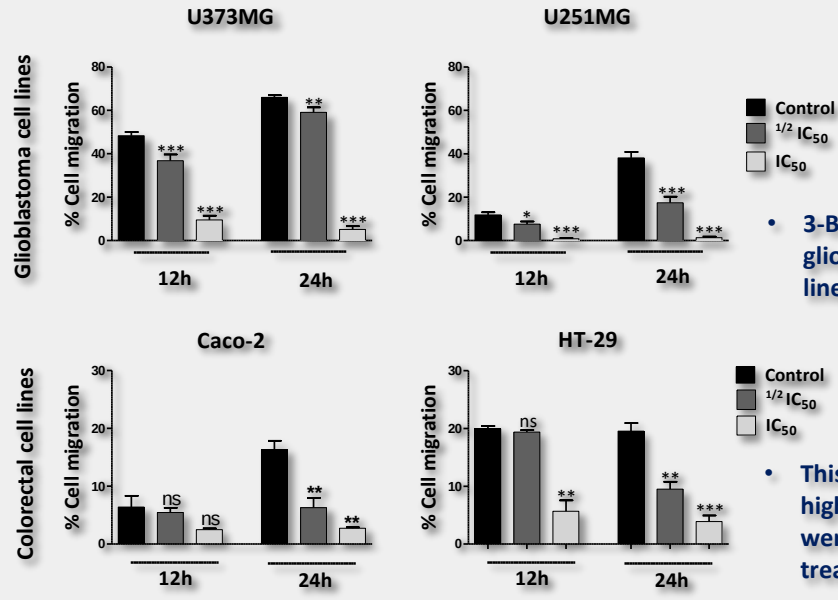


Figure 1. Quantification of the extracellular glucose and lactate in different cancer cell lines. Significantly different between groups: *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; ns: no statistically significant compared to control.

- 3-BP treatment induced a decrease of glucose consumption and lactate production in all cell lines.

Cell migration capacity in glioblastoma and colorectal cancer cell lines



- 3-BP inhibits migratory capacity of both glioblastoma and colorectal cancer cell lines.
- This effect was more evident when higher concentrations of the compound were used, namely after 24 hours of treatment.

Figure 4. Effect of 3-BP in migration capacity in different cancer cell lines. Significantly different between groups: *P < 0.1;**P < 0.01; ***P < 0.001; ****P < 0.0001; ns: no statistically significant compared to control.

MCTs expression in glioblastoma and colorectal cancer cell lines

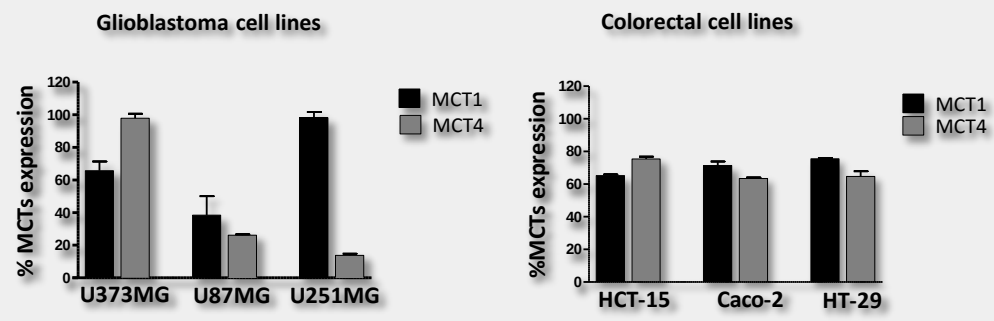
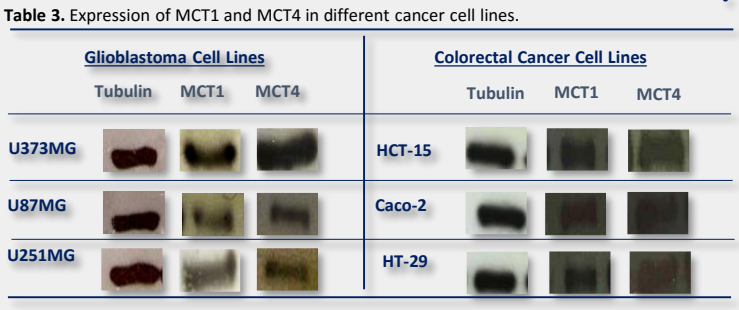


Figure 5. Expression profile of MCT1 and MCT4 in different cancer cell lines.

Table 3. Expression of MCT1 and MCT4 in different cancer cell lines.



- In glioblastoma cell lines, MCT4 is more expressed in the most sensitive cell line, U373MG. MCT1 is more expressed in U251MG and U373MG cell lines.
- In colorectal, there was no relevant differences between cell lines in MCTs expression.